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Aberrations of anterior insular cortex functional connectivity in nontreatment-seeking alcoholics

Meredith E Halcomb[a,b], Evgeny J Chumin[a,b,c], Joaquín Goñi[d], Mario Dzemidzic[a,b],

Karmen K Yoder*[a,b,c,f]

[a]Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, USA

[b]Indiana University Center for Neuroimaging, Indiana University School of Medicine, Indianapolis, IN, USA

[c]Stark Neurosciences Research Institution, Indiana University School of Medicine, Indiananpolis, IN, USA

[d]Purdue Institute for Integrative Neuroscience, Purdue University, West-Lafayette, IN, USA.

[e]Department of Neurology, Indiana University School of Medicine, Indianapolis, IN USA

[f]Department of Psychology, Indiana University Purdue University Indianapolis, Indianapolis, IN, USA

*Corresponding Author
Karmen K. Yoder, Ph.D.
Ph: 317.963.7507
Fx: 317.963.7547
kkyoder@iupui.edu

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Abstract

An emergent literature suggests that resting state functional magnetic resonance imaging (rsfMRI) functional connectivity (FC) patterns are aberrant in alcohol use disorder (AUD) populations. The salience network (SAL) is an established set of brain regions prominent in salience attribution and valuation, and includes the anterior insular cortex (AIC). The SAL is thought to play a role in AUD through directing increased attention to interoceptive cues of intoxication. There is very little information on the salience network (SAL) in AUD, and, in particular, there are no data on SAL FC in currently drinking, nontreatment seeking individuals with AUD (NTS). rsfMRI data from 16 NTS and 21 social drinkers (SD) were compared using FC correlation maps from ten seed regions of interest in the bilateral AIC. As anticipated, SD subjects demonstrated greater insular FC with frontal and parietal regions. We also found that, compared to SD, NTS had higher insular FC with hippocampal and medial orbitofrontal regions. The apparent overactivity in brain networks involved in salience, learning, and behavioral control in NTS suggests possible mechanisms in the development and maintenance of AUD.

Keywords: alcohol use disorder, resting state functional magnetic resonance imaging, salience network, interoception, alcoholism, hippocampus, orbitofrontal cortex

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1. Introduction

Heavy alcohol consumption can result in maladaptive behaviors and physiological damage and is a prevalent, debilitating disorder. Recent research estimates that, within a twelve-month period, approximately 13.9% of the United States population met the Diagnostic and Statistical Manual version V (DSM-5) criteria for diagnosis of alcohol use disorder (AUD) (Grant et al., 2015; Stahre et al., 2014). Decades of preclinical investigation have pointed to numerous neural mechanisms and pathways implicated in the development and maintenance of AUD. With the advent of advanced neuroimaging techniques, we can now examine how the disorder manifests *in vivo*. Although evidence from neuroimaging indicates aberrant structural integrity and functional activity in AUD populations (Chumin et al., 2018; Courtney et al., 2013; Fortier et al., 2014; Rogers et al., 2012; Schacht et al., 2013; Schulte et al., 2010; Wang et al., 1993), there is still much to learn about how specific circuitry is altered in AUD.

Recent advances in functional magnetic resonance imaging (fMRI) acquisition and analysis have greatly enhanced our ability to study critical brain systems involved in AUD. In particular, functional connectivity (FC) analysis of fMRI data can quantify the coherence and strength of functional connections between distinct brain regions (Beckmann et al., 2005; Fox et al., 2005; Greicius et al., 2003). FC is often evaluated during a “resting state”, while the subject is lying still in the scanner, without any task being performed. These task-free fMRI data can be used to extract resting state networks (RSNs), which consist of brain regions that are temporally – and presumably functionally -- coupled (Greicius, 2008; Greicius et al., 2003; Lowe et al., 2000). There is now an emergent literature on alterations in FC within the AUD population, although

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most of the work has been done in abstinent individuals (Camchong et al., 2013; Chanraud et al., 2011; Jansen et al., 2015; Müller-Oehring et al., 2015; Sjoerds et al., 2017; Zhu et al., 2015). There are only a few studies that report findings in either at-risk drinkers (Vergara et al., 2017) or currently drinking alcohol-dependent subjects (Weiland et al., 2014; Zhu et al., 2015), albeit with equivocal results. Weiland et al. (2014) found that currently-drinking AUD individuals had widespread lower FC compared to controls, whereas Zhu et al. (2015) detected higher FC in multiple networks. Although the Vergara (2017) study did not focus on AUD per se, one of their findings was lower FC in the insula in at-risk alcohol drinkers, supportive of our laboratory's interest in the potential role of the salience network in alcoholism.

The salience network (SAL) is defined as a functional circuit comprised of the dorsal anterior cingulate cortex (ACC), portions of the anterior insular cortex (AIC), and medial prefrontal cortex (Seeley et al., 2007). The SAL mediates interoceptive awareness and attentional shifts toward relevant (salient) internal and external stimuli. As part of this perceptual role, it is responsible for assigning valence and relative value strength to emotionally relevant sensory information. Ultimately, the SAL interacts with other brain networks to direct attention and modulate behavioral output, including motivated behaviors (Goulden et al., 2014; Menon and Uddin, 2010; Zhou et al., 2018). Given that alcohol dependence involves seeking out and obtaining alcohol (i.e., motivated behaviors) despite adverse consequences, we hypothesized that the SAL may have altered function in currently-drinking individuals with AUD.

There are also numerous studies that support a role for the insula in addictive behaviors. For example, drug-abstinent populations (including addictions to nicotine,

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cocaine and alcohol) have higher cue-induced insular activation (Kilts et al., 2004; McBride et al., 2006; Myrick et al., 2003; Tapert et al., 2004). Greater insular activation was also noted in a group of alcohol-dependent women during a task involving alcohol cues and the likelihood to drink despite negative outcomes (Arcurio et al., 2015). Human studies and preclinical work both show that lesions of the insula mitigate drug seeking behaviors (Contreras et al., 2007; Naqvi et al., 2007), suggesting that the insula is integral to the development and maintenance of alcohol and substance use disorders.

At present, there is no information regarding the FC of the insula to other brain regions in currently drinking, non-treatment-seeking (NTS) alcoholics. Given the prominent role of the AIC in the salience network and its potential influence in drug-seeking behaviors, detecting aberrations of insular FC in an NTS sample would greatly enhance our understanding of how insular mechanisms may mediate drinking behavior in AUD. Thus, our primary goal was to perform a whole-brain resting state FC study in NTS subjects and social drinkers (SD), using seeds placed in the AIC. Because insular function has been documented to be lateralized (Cauda et al., 2011; Duerden et al., 2013; Sander and Scheich, 2005), we evaluated functional connectivity of the left and right anterior insula separately. We hypothesized that connectivity between the AIC and regions associated with reward processing, salience attribution, and behavioral control would be altered in NTS. The potential impact of this work lies in the possibility that, if the functional connectivity of the salience network is altered in AUD, it may be feasible to develop neuroimaging protocols that will utilize SAL function as a marker to predict treatment response and risk for relapse. Additionally, such protocols could be used to

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track longitudinal changes in the SAL, which could lead to testing existing and future therapies for alcoholism for the ability to modulate SAL function.

2. Methods

2.1 Subjects

Study procedures were approved by the Indiana University Institutional Review Board. Informed consent from each subject was obtained upon determination that breath alcohol concentration was 0 mg%. Seventeen non-treatment-seeking alcoholics (NTS) and 22 social drinkers (SD) completed the study protocol. NTS subjects met DSM-IV criteria for alcohol dependence as determined by the Semi-structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994). NTS subjects were not actively seeking treatment and had not sought treatment within the last year. Exclusionary criteria included: contraindications for MRI, positive urine pregnancy screen, positive urine toxicology screen for illicit substances (except marijuana), current use of any psychotropic medication, history or presence of any neurological, psychiatric disorders (including substance abuse or dependence), or major medical disorders. Demographics and drinking characteristics are listed in Table 1. Supplemental Materials Table 1 lists the percentage of subjects who reported any prior substance use during the course of their lifetime.

2.2 General Study Procedures

The MRI data reported herein were acquired as part of two separate PET protocols. MRI acquisition sequences were identical for all subjects. MRIs were completed in the morning prior to any subsequent experimental procedures. Both PET

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studies included NTS and SD subjects according to the criteria described above. Upon subjects' arrival, sobriety was confirmed by measurements of breath alcohol concentration (BrAC) with an Alcotest 6510 (Dräger Manufacturing, Irving, TX). Urine samples were collected for drug and pregnancy testing. The Clinical Withdrawal Assessment for Alcohol, Revised (CIWA-AR) was administered to NTS subjects periodically to monitor for alcohol withdrawal. (Sullivan et al., 1989). Subjects underwent a 90 minute magnetic resonance imaging session, which started with a high resolution anatomical scan followed by a 10 minute resting state functional MRI scan as detailed below. Subjects were instructed to lay still with their eyes closed and to keep their mind clear. All subjects reported after the scan that they had remained awake and complied with all instructions.

2.3 Image Acquisition

Imaging was performed on a Siemens 3T Prisma (Erlangen, Germany) using a 64-channel head coil array. A high-resolution anatomic volume (3D magnetization prepared rapid gradient echo (MP-RAGE); 1.05 × 1.05 × 1.2 mm^3 or 1.00 × 1.00 × 1.00 mm^3 voxels) was used for positioning the blood oxygenation level dependent (BOLD) resting state data acquisition. Five hundred whole-brain BOLD volumes were acquired using a multiband (MB) echo-planar imaging (EPI) sequence (Center for Magnetic Resonance Research at the University of Minnesota, gradient echo, repetition/echo time TR/TE=1200/29 ms, flip angle 65º, field-of-view 220 × 220 mm, matrix 88 × 88, 54 2.5 mm slices, 2.5 × 2.5 × 2.5 mm^3 voxel, slice acceleration factor = 3) (Smith et al., 2013). The first 25 seconds of each resting state scan were excluded to ensure that only volumes with steady state magnetization would be considered for statistical inferences.

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BOLD acquisition was preceded by a pair of MB=3 spin echo field mapping scans (3 A-P and 3 P-A phase direction volumes, TR=1560 ms, TE = 49.80 ms) with imaging and voxel sizes identical to the BOLD acquisition.

2.4 Image Preprocessing

Preprocessing was performed with a Matlab-based processing pipeline using FMRIB Software Library (FSL version 5.0.9) (Smith et al., 2004) as detailed in (Amico et al., 2017; Contreras et al., 2017). Resting state functional connectivity data were processed in native BOLD EPI space. T1-weighted MP-RAGE volumes were denoised prior to brain masking and extraction (FSL “bet”) and then nonlinearly transformed (FSL’s FLIRT and FNIRT) to the Montreal Neurological Institute (MNI) brain template space. Image processing included BOLD volume unwarping using FSL's topup/applytopup (using spin echo field mapping scans), slice acquisition timing correction, head motion realignment, registration to T1, demeaning and detrending, band pass filtering (0.009-0.08 Hz), and normalization to mode 1000.

Based upon Power et al.’s approach, motion regressors from the realignment and their derivatives were regressed out (Power et al., 2012; Power et al., 2014). We used 3 image quality control metrics (frame displacement, “DVARS” [D refers to the temporal derivative of BOLD time courses, VARS refers to RMS variance over voxels], and whole-brain standard deviation of BOLD signal) to identify and exclude (“scrub”) outlier BOLD volumes. Outlier cutoffs were set at 0.3 mm for frame displacement (characterized as medium head motion) and for 1.7 standardized DVARS (Nichols, 2017; Parker et al., 2017; Power et al., 2015). Any BOLD volume defined as an outlier

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was “scrubbed” from statistical inferences by excluding it from the calculation of correlation coefficients. There were no significant differences between groups in frame displacement, DVARS, or percentage volumes scrubbed (Supplementary Figure 1). In addition, two subjects whose scans had >50% outlier volumes were dropped from the analyses (one SD subject and one NTS subject); thus, the final dataset was comprised of 21 SD and 16 NTS subjects. Principal component analysis (PCA) regression was performed with the Matlab *pca* function after band-pass filtering so that all components were within the frequency range of interest (Chai et al., 2012; Power et al., 2015) . The first five signals obtained by PCA from each of the three tissue compartments were regressed out to address confounding effects of physiologic noise and residual head motion within eroded masks of the whole-brain gray matter, white matter and cerebrospinal fluid of the lateral ventricles. This included global signal regression (Power et al., 2012; Power et al., 2015).

2.5 Regions of Interest and Functional Connectivity

A priori insular seed regions of interest (ROIs) were created using the MarsBar tooolbox in SPM12 (Brett et al., 2002). The 5 × 5 × 5 mm^3 cubical seeds were based upon previous characterization of insular FC (Cauda et al., 2012; Cauda et al., 2011). We selected a subset of the Cauda seeds (“2”, ”5”, “6”, ”8”, and “9”, according to their nomenclature; Table 2) within the anterior insular cortex (AIC), which is a node of the salience network (Figure 1). Seed regions were created in the 1 mm isotopic voxel MNI space and were nonlinearly transformed to native space of each subject. Mean BOLD time series from each seed region was extracted and correlated with time series of all voxels to create whole-brain Pearson’s correlation coefficient maps. Then, a variance-

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stabilizing Fisher z-transformation was performed to create a *Z*-statistic map. Native space *Z*-statistic maps of each subject were inversely transformed to MNI space using FSL's registration and smoothed by a 6 mm full width at a half maximum Gaussian kernel.

2.6 Statistics

Independent *t*-tests were performed to test for group differences in age, education, and recent drinking metrics (drinks consumed per drinking day and drinks consumed per week). Chi-squared tests were used to assess group differences in the ratios of sex and smoking status.

Ten *Z*-statistic images of each subject (one for each seed) were entered into an SPM12 group random effects factorial model, with Group(2; NTS, SD) and Seed(10; 5 seeds × 2 hemispheres) treated as fixed effects. Group was modeled as an independent factor, while seed was a dependent factor. Contrasts were specified to yield "average" connectivity of left seeds, right seeds, or all seeds (main effect). The design matrix presented in Supplementary Figure 2 illustrates the covariance structure of the model, which reflects the repeated-measures nature of the data (with 10 statistical maps contributed by each subject). We tested for group differences in the whole-brain connectivity maps using the SD > NTS and NTS > SD contrasts. Statistical significance was inferred at the cluster level ($p_{FWE} < 0.05$) using family wise error (FWE) correction for multiple comparisons across the whole brain, with a cluster-forming height threshold set to $p = 0.001$, uncorrected (Eklund et al., 2016). To simplify the interpretation and avoid "the double subtraction pitfall," we reported significant group differences in FC only in areas that showed positive FC with the seed region. This

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allowed us to exclude areas that were not associated or were anticorrelated with the seed regions. Specifically, SD>NTS findings were constrained by an inclusive mask from the positive SD contrast (SD subjects only; voxel-wise $p<0.05$, uncorrected). Similarly, the NTS positive contrast was used as an inclusive mask when reporting NTS>SD results. Analyses were conducted both with and without sex, age, and education as covariates.

We also examined an SPM multiple regression model across all subjects that included recent drinking as indexed by number of drinks consumed per week. This measure of alcohol intake was chosen as it better describes overall drinking patterns relative to drinks consumed per drinking day. The dependent variable in these regression models were each subject's Z-statistic connectivity map (entered into the model as described above; left and right seeds were averaged for each side and were and tested indepedently within the model).

3. Results

3.1 Demographics

There were no significant group differences in sex, age, smoking status or education. As anticipated, groups differed on drinks per drinking day (Dr/DD) and drinks per week (Dr/Wk), with the NTS consuming significantly greater amounts of alcohol, *p*s < 0.001.

3.2 Functional Connectivity Patterns Across All Subjects

For both the left and right AIC, the main effects of insular FC across all subjects were similar to previously reported findings (Cauda et al., 2011). Conjunction analyses

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confirmed significant overlap in FC in both SD and NTS subjects (see Supplementary Material, Figure 3). There was significant connectivity between the AIC and other nodes of the SAL, the cerebellum, subcortical regions, and the prefrontal cortex (see Supplementary Material Figures 3 and 5; Tables 2 and 3). Below, we present the group differences from the right and left AIC seed analyses. All reported group differences remained significant when age, sex, and education were included as covariates.

3.3 Between Group Differences

Significant differences in the patterns of AIC FC were present between NTS and SD subjects. Overall, SD subjects demonstrated greater FC than NTS, mostly with frontal and parietal regions from both left and right hemisphere AIC seed regions (Table 3). However, NTS subjects had greater FC than SD in temporal, limbic, and orbitofrontal regions (Table 4).

3.4 SD > NTS Contrast

Subjects in the SD group displayed greater bilateral FC with frontoparietal regions, including the right superior orbital gyrus (SOG) and the right inferior frontal gyrus (IFG) (Figures 3 and 4A). The left AIC seeds showed an enhanced FC with the right superior parietal lobule (SPL), left calcarine gyrus (CalG), and the left inferior frontal gyrus (Figure 3). The right AIC seeds showed greater FC with the posterior cerebellum, the left superior parietal lobule (SPL), and the right putamen (PUT) (Figures 3 and 4B).

3.5 NTS > SD Contrast

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Compared to SD, NTS subjects had greater insular connectivity with several regions (Figure 3; Table 4). The left AIC seeds had greater FC with the anterior cerebellum, the right fusiform gyrus (FFG), and the left posterior insular cortex (PIC). Seeds located in the right hemisphere displayed greater FC with bilateral parahippocampal gyri (PHG; Figure 4A), medial orbitofrontal cortex (mOFC; Figure 4B), the left hippocampus (HIP; Figure 4A), and the left supramarginal gyrus (SMG).

3.6 Alcohol intake correlations

When multiple regression models were corrected for multiple comparisons across all subjects, there were no associations between insular FC and recent alcohol intake. This finding, however, was not unexpected. First, drinking rates were self-reported, which can be inherently unreliable. There was also high variability in the metric of drinks per week in both groups. Both factors could have contributed to a lack of a relationship between FC and drinking behavior.

4. Discussion

This study was the first to detect significant differences in FC related to the salience network (SAL) in an NTS sample. Overall, there were disparate patterns of insular connectivity between NTS and SD subjects, which occurred in regions associated with memory formation (Miendlarzewska et al., 2016; Zola-Morgan et al., 1989), stimulus valuation, reinforcement, and decision making (Kringelbach and Rolls, 2004). The SAL integrates information about interoceptive sensations and perceptions about external stimuli, and assigns valence to these experiences (Craig, 2010; Naqvi and Bechara, 2010; Seeley et al., 2007). Additional work suggests that the insula

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provides representations of emotional states linked to stimuli, as well as any cues associated with the stimuli (Craig and Craig, 2009; Gasquoine, 2014; Wager and Barrett, 2017). The present findings suggest that an insular mechanism may be involved in the maintenance of hazardous drinking behavior in currently-drinking AUD individuals, perhaps through exaggerated attribution of positive valence to intoxication and/or cues associated with alcohol consumption.

4.1 Greater Insular FC in NTS

In the NTS sample, there was greater insular FC with bilateral hippocampal regions, which are closely associated with memory formation and encoding. There is abundant evidence for alterations in the hippocampal regions of subjects with long-term alcohol exposure (Livy et al., 2003; Riley and Walker, 1978; Zhou et al., 2011). As with other types of learning, the hippocampus is involved with the short- and long-term memory formation necessary for drug-associated learning (Jones and Bonci, 2005). Therefore, it is possible that the higher insular-hippocampal connectivity observed in our NTS sample could have real-world consequences. For example, greater FC between the AIC and bilateral hippocampal regions in the NTS group could indicate a tendency for formation of abnormally strong associations with alcohol-related interoceptive states (e.g., intoxication) and/or the cues associated with drinking. If the memory of intoxication is represented more intensely or with more positive valence, then this could exert greater influence over behaviors such as alcohol seeking and consumption, relative to representations such as the negative consequences of alcohol consumption. Thus, a disproportionate assignment of positive value to alcohol-related interoception

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and associated environmental cues could contribute to the sustained alcohol use in NTS populations.

NTS subjects also displayed greater FC between the right AIC and the medial OFC (mOFC), a region involved with reward valuation and encoding (Elliott et al., 2000; Kringelbach and Rolls, 2004; O'Doherty et al., 2001; Sescousse et al., 2010), as well as decision-making based on expected outcomes of actions (Schoenbaum et al., 2006). If the AIC and mOFC are working in synchrony, it is possible that the mOFC may be compromised in its ability to modulate behavioral decisions based on the skewed reward value of alcohol and associated environmental stimuli. This could also help explain why individuals with AUD continue to drink despite negative outcomes.

The present results may be counter-intuitive in the face of other neuroimaging data that suggests that addicts have lower OFC function (Bolla et al., 2003; London et al., 2000; Schoenbaum and Roesch, 2005), and preclinical data that indicate that drugs of abuse compromise the ability of the OFC to modulate behavior and learning (for review, Schoenbaum et al., 2006). However, it is consistent with overactivation of the OFC observed in drug addicts during exposure to drug-related cues (Goldstein et al., 2007; London et al., 2000; Tomasi et al., 2015). It should be noted that this is the first study in a non-abstinent AUD population.

4.2 Lower FC in NTS

Relative to SD, NTS subjects had lower insular connectivity with prefrontal nodes of the frontoparietal attention network (FPN). The FPN is considered a “flexible hub” of cognitive control (Cole et al., 2013), and is essential in driving goal-directed behavior

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(Cole et al., 2014). Given the extensive influence of the FPN on controlling behavior, degradation of this system could lead to an inability to regulate behavior. Thus, lower FPN–AIC connectivity in NTS may inhibit cognitive and behavioral control over alcohol consumption despite recurrent negative consequences.

Connectivity between the insula and putamen has been confirmed in healthy populations (Cauda et al., 2011; Di Martino et al., 2008; Postuma and Dagher, 2006). NTS subjects displayed lower right insular FC with the right anterior caudate and anterior putamen as compared to SD subjects. This is consistent with previous research in cocaine addicts (McHugh et al., 2013). The caudate and putamen are integral components of the basal ganglia, and contribute to both motor and cognitive processes (DeLong et al., 1984; Marchand et al., 2008; Packard and Knowlton, 2002; Seger and Cincotta, 2005; White, 2009). The putamen in particular is heavily implicated in mediating habit formation, a prominent aspect of AUD (Tricomi et al., 2009; Yin and Knowlton, 2006). The diminished insular-anterior striatal FC observed here may suggest potential alterations in anterior putamen function in NTS subjects that could contribute to habitual alcohol consumption.

The primary limitation of this study is a modest sample size. Although we found significant results, a larger sample could permit us to detect more discrete regions that may have compromised insular connectivity in AUD. The sample size also did not permit us to test for effects of sex, age, or smoking status. While we utilized an *a priori* seed-based approach, whole-brain connectomics approaches may provide more comprehensive information about functional abnormalities in AUD. Although no subjects endorsed falling asleep during the scan, it is possible they could have briefly fallen

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asleep without being aware this occurred. Future studies could implement the use of the Stanford Sleepiness Scale to evaluate states of consciousness immediately prior to and after resting state scans to gain insight into the subjects' level of consciousness and its changes during imaging (Hoddes et al., 1973; Vaghi et al., 2017). We also cannot make any assumptions about the neurochemistry of the neuronal populations that are contributing to the BOLD signal. Finally, there are conceptual limitations with our explanation of the data. It could be the case that the observed differences in the SAL are independent of drinking behaviors, and may instead reflect a broader, non-specific effect of years of chronic drinking on insular circuitry in AUD. Alternatively, such differences could reflect premorbid differences in FC that could be related to risk factors for development of AUD. Carefully planned longitudinal studies would be required to disentangle these possible interpretations.

In summary, a mounting body of evidence strongly indicates alterations in overall FC in AUD individuals. The results extend previous findings to include information regarding insular connectivity patterns in currently-drinking AUD subjects. Together, This research illustrates a potential impact of the insula (and by extension, the salience network) on addictive behaviors. Future work with task-based fMRI will be needed to test specific hypotheses about how neural circuits that mediate salience attribution, behavioral control, and habit formation are altered in AUD.

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Contributors

KKY designed the study; secured all regulatory approvals; obtained funding for the study; supervised all aspects of the study, including but not limited to, subject recruitment, data acquisition and processing, and statistical analysis; contributed to the intellectual content of the manuscript; and approved the final version of the manuscript.

MEH assisted with subject recruitment and study day execution; conducted all image processing and quality assurance procedures; conducted the statistical analyses; and provided the initial drafts of the manuscript.

EJC helped build, test, and validate the in-house imaging pipeline; supervised the data processing and QA and contributed to the intellectual content of the manuscipt.

JG initiated the in-house pipeline components; supervised the data processing and QA; and contributed to the intellectural content of the manuscript.

MD designed the MRI sequences; supervised all data acquisition, processing, and QA; assisted with the design of the statistical analysis; and contributed intellectual content to the manuscript.

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Conflicts of interest: None of the authors have any conflicts of interest to declare

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Table 1. Subject Demographics and Characteristics

	SD (*n* = 21)	**NTS (*n* = 16)**	**Significance**
Sex	10 M; 11 F	11 M; 5 F	$X^2(1) = 1.52$, $p = 0.32$
Age	35.3 (± 11.4)	37.3 (± 11.6)	$t(35) = -0.32$, $p = 0.60$
Edu	15.6 (± 2.1)	14.1 (± 2.5)	$t(35) = 1.97$, $p = 0.06$
Race	17 W; 3 AA; 1 As	12 W; 3 AA; 1 AI	
Drinks/day	2.5 (± 0.8)	5.7 (± 3.5)	$t(35) = -4.00$, $p < 0.001$
Drinks/week	8.7 (± 5.8)	30.0 (± 22.0)	$t(35) = -4.078$, $p < 0.001$
Smokers	4	6	$X^2(1) = 1.57$, $p = 0.274$

M: Male; F: Female; *n.s.*: non-significant; W: White; AA: African American; As: Asian; AI: American Indian.

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Table 2. MNI Coordinates for Anterior Insular Cortex Seed Regions

ROI	x	y	z	mm^3
2 L	-37	8	-7	125
2 R	40	9	-8	125
5 L	-33	12	4	125
5 R	38	10	0	125
6 L	-39	2	1	125
6 R	40	2	0	125
8 L	-31	12	9	125
8 R	34	11	8	125
9 L	-36	1	8	125
9 R	36	0	7	125

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Table 3. Regions with greater FC in SD than NTS (SD > NTS Contrasts)

LEFT AIC SEEDS					**RIGHT AIC SEEDS**				
Region	**Cluster**		**Peak**		**Region**	**Cluster**		**Peak**	
	k	***p*(FWE)**	***Z***	**MNI Coordinate**		***k***	***p*(FWE)**	***Z***	**MNI Coordinate**
R Superior orbital gyrus	5169	<0.001	6.26	31 65 -3	*L Cerbellum*	3349	<0.001	4.62	-8 -75 -16
R Superior parietal lobule	2578	<0.001	5.69	43 -50 70	*L Superior parietal lobule*	1918	0.003	4.97	-45 -50 66
L Calcarine gyrus	1798	0.005	4.26	-11 -67 6	*R Inferior frontal gyrus*	1368	0.019	4.69	36 33 28
L Inferior frontal gyrus	1411	0.017	4.64	-57 32 11	*R Putamen*	1343	0.021	4.46	21 7 11
R Inferior frontal gyrus	1251	0.029	4.14	48 16 14	*R Superior Orbital gyrus*	1148	0.042	4.14	29 65 -4

Statistical inferences made using cluster-level significance $p_{FWE} < 0.05$, FWE-corrected for the whole-brain multiple comparisons with the cluster-forming threshold, $p = 0.001$, uncorrected.

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Table 4. Regions with greater FC in NTS than SD (NTS > SD Contrasts)

LEFT AIC SEEDS					**RIGHT AIC SEEDS**				
Region	**Cluster**		**Peak**		**Region**	**Cluster**		**Peak**	
	k	***p* (FWE)**	***Z***	**MNI Coordinates**		***k***	***p*(FWE)**	***Z***	**MNI Coordinates**
L Cerebellum	2958	< 0.001	4.68	-21 -36 -28	*R medial OFC*	2437	0.001	5.12	2 32 -25
R Fusiform Gyrus	2096	0.002	5.34	33 -56 -10	*L Hippocampus*	1807	0.004	4.70	-34 -16 -19
R Posterior insular cortex	1187	0.037	4.52	34 -25 20	*L Supramarginal gyrus*	1439	0.015	4.73	-70 -21 15
					R Parahippocampal gyrus	1334	0.022	5.72	19 -7 -26

Statistical inferences made using cluster-level significance $p_{FWE} < 0.05$, FWE-corrected for the whole-brain multiple comparisons with the cluster-forming threshold, $p = 0.001$, uncorrected.

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Figure 1. Anterior insula seed regions. 5mm^3 cubical seed regions are shown in an axial view of the right hemisphere (R). Coordinates denote the centroid of each seed, and are in MNI space. Numerical designations for each seed are from Cauda et al. 2011, 2012.

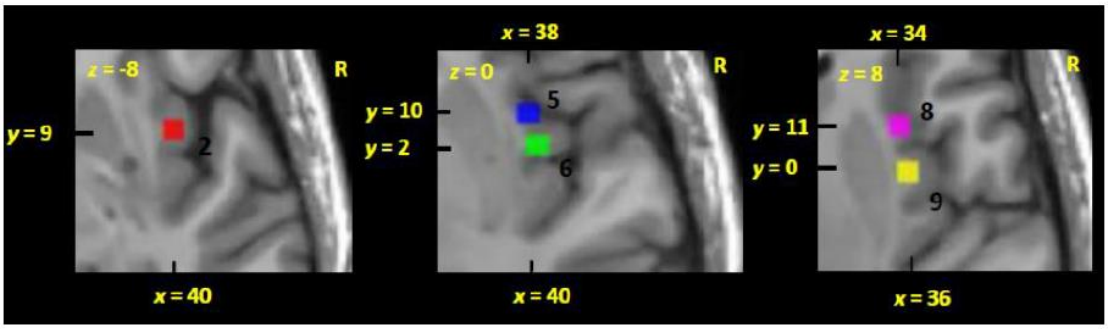

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Figure 2. Functional connectivity patterns of the left and right anterior insula seeds (panels A and B, respectively). Regions in which SD have greater FC compared to NTS are shown in magenta, while regions where NTS have greater FC than SD are indicated in cyan. Statistical inferences were made with a cluster-level significance $p_{FWE} < 0.05$, FWE-corrected for the whole-brain multiple comparisons with the cluster-forming threshold, $p = 0.001$, uncorrected.

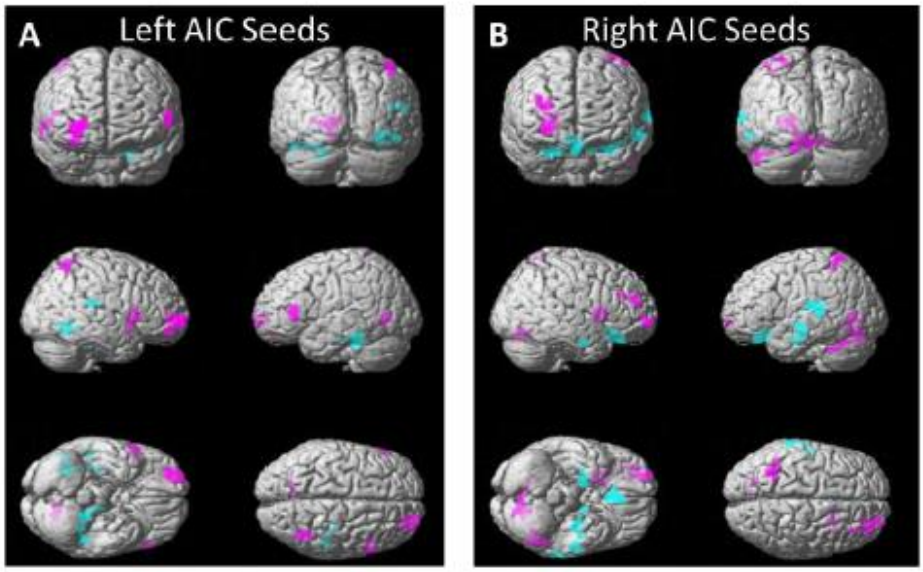

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Figure 3. Additional regions in which insular FC is greater in SD than in NTS. The left AIC seeds had enhanced FC with the right superior orbital gyrus (SOG) (Panel A; sagittal view). The right AIC seeds had higher FC with the right anterior putamen (Panel B; coronal view). Statistical inferences were made with a cluster-level significance $p_{FWE} < 0.05$ (FWE-corrected for the whole-brain multiple comparisons with the cluster-forming threshold, $p = 0.001$, uncorrected).

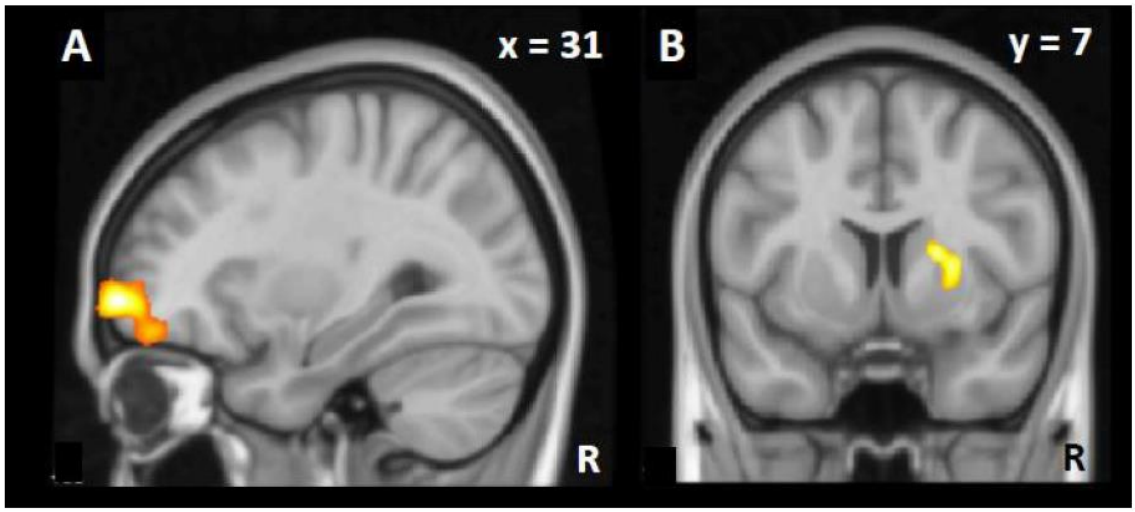

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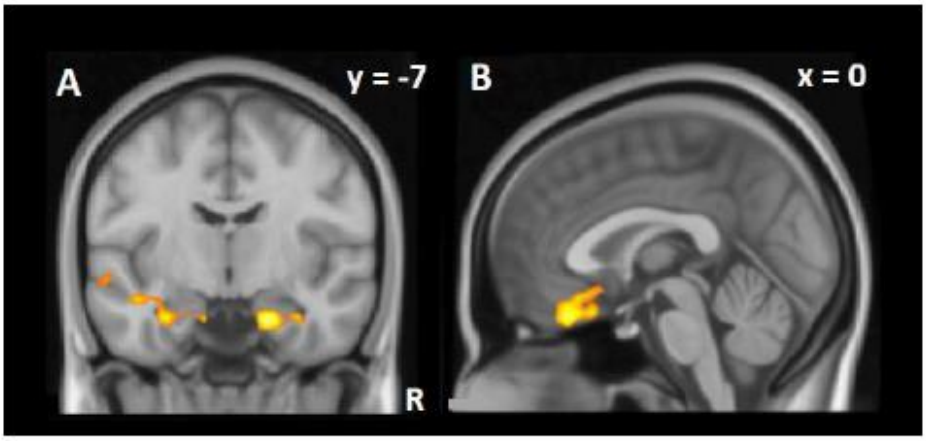
Figure 4. Additional regions with greater FC in NTS than SD. Data in both panels are from the right AIC seed analysis. NTS have higher insular connectivity with bilateral hippocampal regions (Panel A; coronal view) as well as the caudal portion of medial OFC (Panel B, midline sagittal view). Statistical inferences were made with a cluster-level significance $p_{FWE} < 0.05$ (FWE-corrected for the whole-brain multiple comparisons with the cluster-forming threshold, $p = 0.001$, uncorrected).



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